Journal of Medical Virology 51:139–144 (1997)

Prevalence of Anti-*Vif* Antibodies in HIV-1 Infected Individuals Assessed Using Recombinant Baculovirus Expressed *Vif* Protein

C. O'Neil, D. Lee, G. Clewley, M.A. Johnson, and V.C. Emery*

Department of Virology and the AIDS Unit, Royal Free Hospital and School of Medicine, London, United Kingdom

A 630 base pair fragment of the HIV-1 genome encompassing the entire *vif* open reading frame has been produced by the polymerase chain reaction and cloned into the baculovirus transfer vector pAcYM1. Extracts from insect cells infected with a recombinant baculovirus expressing the HIV-1 *vif* gene product were used in a radioimmunoassay to analyse 238 sera from HIV infected individuals for the presence of anti-*vif* antibodies. The overall prevalence of anti-*vif* antibodies in this group of patients was 25.3%. Stratification of the group according to CD4 levels showed that anti-*vif* antibodies were more prevalent in patients with CD4 counts below the median of the group (155×10^6 cells/L; $P = 0.005$). A significant increase in anti-*vif* antibodies was observed in patients with CD4 levels less than 280×10^6 cells/L ($P < 0.01$) and in patients with symptomatic HIV infection ($P = 0.0003$). However, there was no significant difference in the prevalence of anti-*vif* antibodies in patients stratified according to p24 antigen status. The implications of these findings in the context of HIV replication are discussed. ***J. Med. Virol. 51:139–144, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: HIV-1; baculovirus; *vif*; radioimmunoassay

INTRODUCTION

The HIV-1 virion infectivity factor (*vif*) encodes a 23 kDa cytoplasmic protein [Lee et al., 1986; Fisher, 1987] which is conserved within the lentiviruses [Oberste and Gonda, 1992]. The *vif* protein has been shown to be essential for efficient virus transmission in cell free infections but dispensable for cell-cell spread of infectious virus [Gabuzda et al., 1992; Sodroski et al., 1986]. The mechanism of action of *vif* has not been fully elucidated although recent data have shown that *vif* is located diffusely throughout the cytoplasm of HIV-1 infected cells [Gonclaves et al., 1994] and may exert its effect at a late stage of virus replication/maturation [Gabuzda et al., 1992; Sakai et al., 1993; Blanc et al., 1993; Hoglund et al., 1994, Borman et al., 1995; von Schwedler et al., 1993].

The HIV-1 *vif* protein is immunogenic in vivo eliciting a weak cytotoxic T lymphocyte response and a humoral immune response [Lamhamedi-Cherruli et al., 1992; Wieland et al., 1990, 1991]. The majority of the B cell immune response to the HIV-1 *vif* protein is directed at two decapeptides comprising the epitopes IEWRKKRY (amino acids 87–94) or DRWNKPQ (amino acids 172–178) [Wieland et al., 1991]. At present, the majority of studies on the prevalence of anti-*vif* antibodies in HIV-1 infected individuals have used prokaryotically expressed *vif* as the antigen source [Schwander et al., 1992; Franchini et al., 1987; Kan et al., 1986]. Frequently the conformational integrity of prokaryotically expressed eukaryotic proteins is questionable and hence recourse to eukaryotic expression systems must be effected. Data generated from prokaryotically expressed *vif* have shown that anti-*vif* antibodies increase in prevalence with decreasing CD4 count and clinical progression [Schwander et al., 1992] although some studies [Kan et al., 1986; Franchini et al., 1987; Ayra and Gallo, 1986; Devash et al., 1990] have found no association between *vif* specific antibodies and stage of HIV disease.

In order to overcome the criticisms of using prokaryotically expressed recombinant *vif* protein as the antigen source for antibody prevalence studies we have elected to use a recombinant baculovirus system to produce the HIV-1 *vif* gene product in insect cells. Since the insect cells perform the variety of post-translational modifications associated with higher eukaryotic systems [reviewed by Kidd and Emery, 1993] the HIV-1 *vif* protein should be comparable to the native protein expressed during HIV-1 replication. We investigated the preva-

*Correspondence to: V.C. Emery, Department of Virology, Royal Free Hospital and School of Medicine, Rowland Hill Street, London NW3 2PF, U.K.

Accepted 1 May 1996

lence of anti-*vif* antibodies using this recombinant antigen and compared the results with the data published previously.

MATERIALS AND METHODS

Insect Cell Culture

Spodoptera frugiperda cells were obtained from AMS Technology (UK) and were cultured as monolayers at 28°C in TC100 medium (Life Technologies Ltd., Paisley, Scotland) supplemented with 10% fetal calf serum (Advanced Protein Products, West Midlands, UK) and penicillin (100 IU/ml) streptomycin (10 μg/ml).

Construction of the Baculovirus Transfer Vector Containing HIV-1 *Vif*

The entire *vif* open reading frame was amplified by polymerase chain reaction (PCR) using the primers CCAAGAAGAAGAAAAGCAAAGATCATT and GATCTCGAAAATCTCCTCGAATTC together with HIV-1 proviral DNA derived from the BH10 strain of HIV-1 (a gift from William James, University of Oxford, UK). PCR was performed under the conditions described in Darlington et al. [1991] to yield the 630 base pair *vif* amplicon. The use of 5′ phosphorylated primers in the PCR enabled the direct cloning of the *vif* amplicon into the baculovirus transfer vector pACYM1 (a gift from D. Bishop, Institute of Virology and Environmental Microbiology, Oxford, UK) at the unique Bam HI restriction endonuclease site, which had been repaired by Klenow fill-in using established methods [Sambrook et al., 1989]. Following transformation of *E. coli* JM109 cells, putative clones containing the *vif* gene were identified by PCR. The orientation of the *vif* gene within the transfer vector was confirmed by DNA sequence analysis using the plasmid sequencing protocols described by Murphy and Kavanagh [1989] and the Sequenase version 2 enzyme according to the manufacturer's instructions.

A clone containing the HIV-1 *vif* gene in the correct orientation relative to the polyhedrin promoter was identified (pAcYM1*vif*) and used for generation of recombinant baculoviruses. Generation of the recombinant *Autographa californica* nucleopolyhedrosis virus (AcNPV) containing the HIV-1 *vif* gene was achieved using the "Baculogold" system according to the manufacturer's instructions (Invitrogen, San Diego, USA). Three days following co-transfection the supernatants were harvested and subjected to plaque assay using semi-confluent monolayers of insect cells as described in King and Possee [1995]. Ten plaques were picked and propagated via subsequent infection of insect cells to increase the viral titres. The titre of the putative recombinant viruses was approximately 6×10^6 pfu/ml. These viruses were used subsequently for analysis of protein expression as described below.

Radiolabelling of Recombinant Proteins

The identification of the HIV-1 *vif* gene product in the recombinant baculovirus infected cells was achieved using pulse labelling with ^{35}S-(L)-methionine as previously described [Matsuura et al., 1987]. Insect cells were infected at a moi of 2–5 with the YM1*vif* recombinant baculovirus and infected cells were harvested at 24 hours and 48 hours postinfection and subjected to SDS PAGE using standard protocols [Sambrook et al., 1989] followed by autoradiography with Hyper film MP (Amersham, Bucks, UK).

Immunofluorescent Staining

Insect cells infected at a moi of 2 were harvested 48 hours postinfection by centrifugation at 2,000*g*. The pellet was resuspended in PBS (500 μl) and 25 ml aliquots were applied to PTFE coated slides. The slides were then dried at room temperature and fixed in acetone at −20°C for 10 minutes. The slides were incubated with a rabbit anti-HIV-1 *vif* antibody (a gift from M. McCrae, University of Warwick, UK) at a dilution of 1:40 in PBS for 30 minutes at 37°C in a humidified chamber. The slides were washed for 10 minutes with PBS and the secondary antibody (mouse anti-rabbit IgG conjugated to fluorescein isothiocyanate) added at a dilution of 1:80 in PBS for 30 minutes at 37°C in a humidified environment. The slides were then washed with PBS for 10 minutes and counterstained with Evans Blue followed by visualisation with laser confocal microscopy.

Detection of Anti-*Vif* Antibodies by Radioimmunoassay (RIA)

Cells infected with a moi of 2 were harvested 72 hours postinfection by centrifugation at 2,000*g* for 10 minutes. The cell pellet was washed with PBS and recentrifuged at 2,000*g* for 10 minutes. The cells were resuspended in 0.25% sodium dodecylsulphate in PBS (w/v) to a concentration of 2×10^7 cells/ml and boiled for 5 minutes. The resulting supernatant was used to coat remova-well plates (Nunc, UK) at a concentration of 10 μg protein/ml in PBS with 100 μl being added to each well. The plates were incubated overnight at 4°C in a humidified chamber. Subsequently, the plates were washed three times with PBS containing Tween 20 (0.05% v/v) and 100 μl of blocking buffer (PBS containing 2% bovine serum albumin fraction 5 [w/v] and 0.5% lactalbumin [w/v]) was added to each well and incubated for 1 hour at room temperature. Following removal of the blocking buffer, 100 μl of either the rabbit anti-HIV-1 *vif* antibody in PBS (1:100 dilution) or 100 μl of each patient's serum (diluted 1:20) in blocking buffer (see above) was added and incubated for 1 hour at 37°C in a humidified container. The plates were then washed six times with PBS containing 0.05% Tween 20 (v/v) and excess liquid was removed. For the detection of the rabbit anti-HIV-1 *vif* antibody 100 μl (2.6 KBq) of ^{125}I labelled anti-rabbit IgG prepared was added to each well whilst for the detection of human sera, 100 μl (2.6KBq) of ^{125}I labelled anti-human IgG antibody was added to each well. The antibodies were iodinated using established methods. The plates were incubated for 1 hour at 37°C in a humidified chamber. The plates were then washed six times with PBS containing 0.25% Tween 20 (v/v) and the bound radioactivity in each well determined using a gamma counter. Binding ratios to test (BRt) and control (BRc)

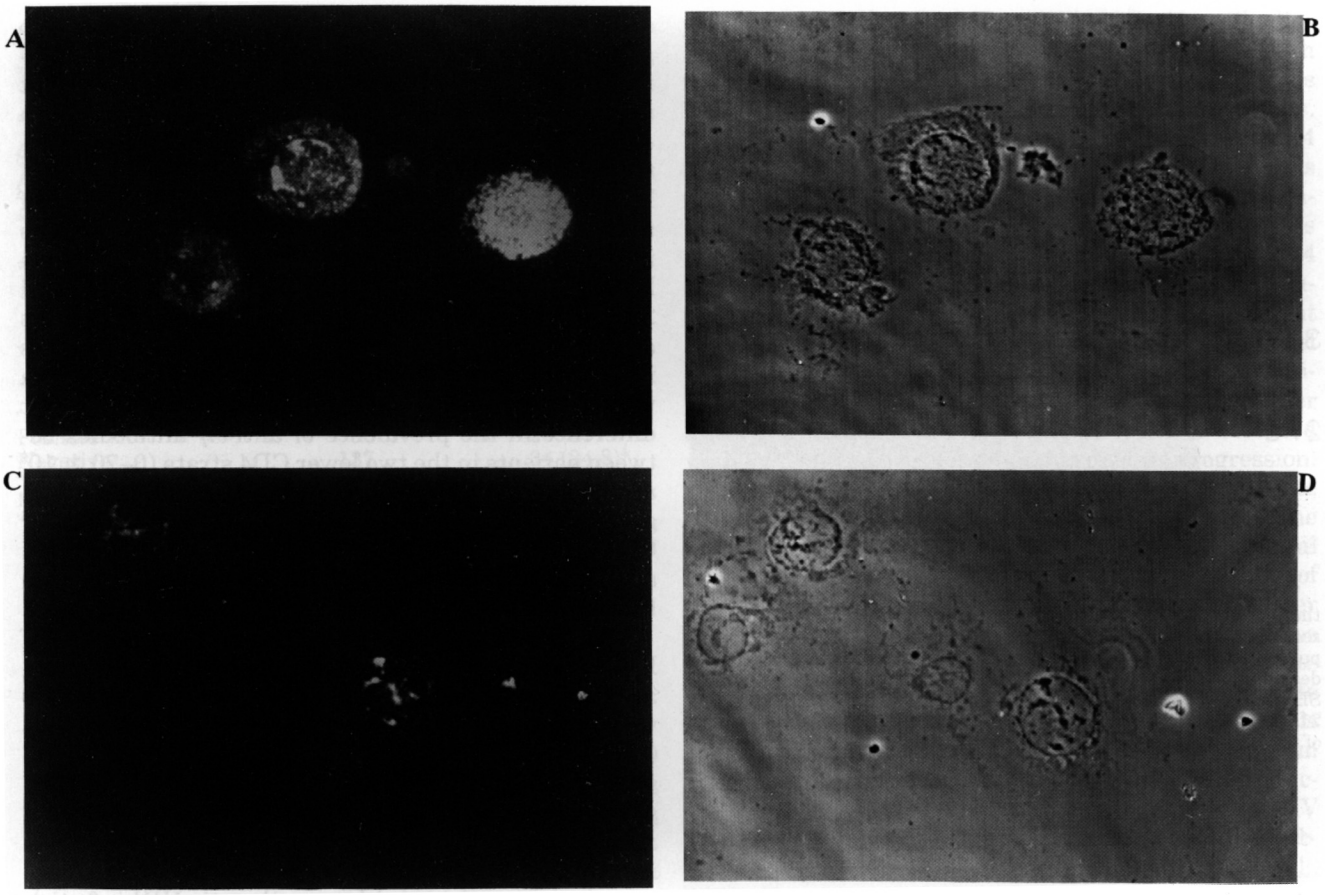

Fig. 1. **A,B:** Immunofluorescence analysis of insect cells infected with the recombinant baculovirus expressing the HIV *vif* product. **A:** Cells were stained with a rabbit anti-*vif* antibody and visualised with an FITC conjugated mouse anti-rabbit IgG second antibody as described in Materials and Methods. **B:** Phase contrast micrograph of the cells shown in *A*. **C:** Immunofluorescence analysis using the rabbit anti-*vif* antibody and insect cells infected with wildtype ACNPV. No specific fluorescence is observed. **D:** Phase contrast micrograph of the cells shown in *C*.

antigens were calculated and a specific binding index (SBI) was determined in which SBI = BRt/BRc. An SBI > 2 was taken to denote the presence of specific antibody. This SBI has been previously used in CMV and HSV RIAs developed at our institution [Berry et al., 1986, 1987].

Detection of HIV-1 p24 Antigen

Immune complex disrupted detection of HIV-1 p24 antigen was achieved using the DuPont HIV-1 p24 ELISA according to the manufacturer's instructions.

RESULTS

Recombinant Baculovirus Expression of *Vif*

The entire HIV-1 *vif* open reading frame was amplified by PCR and cloned into the baculovirus transfer vector PAcYM1. The recombinant baculoviruses (YM1 *vif*) derived by co-transfection of insect cells with Baculogold DNA were assessed for expression of the HIV-1 *vif* protein by ^{35}S-methionine labelling and immunofluorescence using a rabbit anti-*vif* antibody raised against a prokaryotically expressed HIV-1 *vif* protein. The results of the immunofluorescence analysis are shown in Figure 1. The insect cells infected with YM1 *vif* show specific cytoplasmic immunofluorescence (Fig. 1A) using the rabbit anti-*vif* antibody whilst cells infected with wild type baculovirus AcNPV show no specific fluorescence (Fig. 1C). Of the 10 putative recombinant plaques isolated following co-transfection all showed fluorescence with the rabbit anti-*vif* antibody (data not shown). Consequently, only one of the recombinants was selected for further analysis.

The kinetics of expression of *vif* was analysed using pulse labelling with ^{35}S-methionine at 24 and 48 hours postinfection. The results of these analyses are shown in Figure 2 and demonstrate that a specific gene product at 23 kDa was apparent at both 24 and 48 hours postinfection in the YM1*vif* infected insect cells. The *vif* product comprises the majority of the newly synthesised protein at 48 hours postinfection. As expected, the kinetics of production are identical to those of the polyhedrin protein expressed by wildtype AcNPV (Figure 2, lanes 3 and 4).

Radioimmunoassay Detection of Anti-*Vif* Antibodies

Insect cells infected with the recombinant *vif* expressing baculovirus were subsequently used to produce a

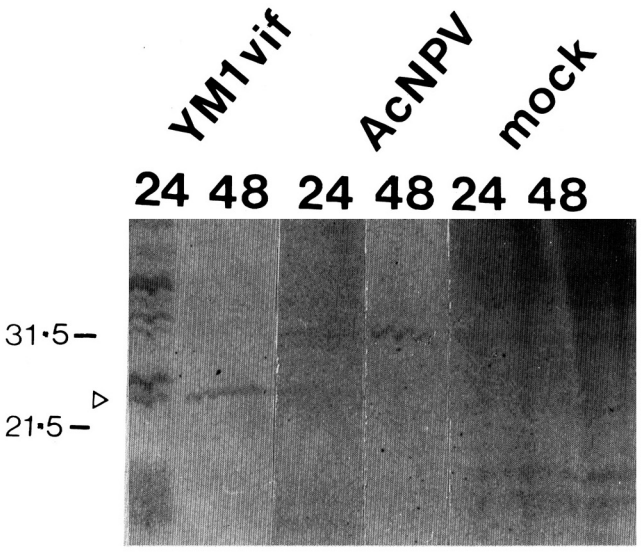

Fig. 2. Time course of expression of the HIV-1 *vif* gene product by the recombinant baculovirus. Insect cells infected with either the *vif* recombinant (YM1*vif*), wildtype AcNPV or mock infected cells were pulse-labelled with ^{35}S methionine at 24 or 48 hours postinfection as described in Materials and Methods. Proteins were analysed by 12% SDS-PAGE followed by autoradiography. Molecular size markers of 21.5 kDa and 31.5 kDa are indicated. The arrow indicates the position of the HIV-1 *vif* gene product which migrates with a Mr of 23 kDa.

TABLE I. Prevalence of Anti-*vif* Antibodies According to CD4 Levels

CD4 level ($\times 10^6$ cells/L)	Number in group	Anti-*vif* positive (%)
0–70	80	27 (33.8)*,***
80–280	78	24 (20.8)*,**
290–1160	80	9 (11.2)**,***

*P = not significant.
**P = 0.004.
***P = 0.0001.

vif specific radioimmunoassay (RIA) for the detection of anti-*vif* antibodies in human sera obtained from patients infected with HIV-1. Various methods to solubilise the *vif* protein were attempted, including freeze-thaw and use of a Triton X-100/NP40 detergent mixture, but these methods failed to produce antigen that could be recognised by the rabbit anti-*vif* antibody in the RIA despite its recognising the *vif* antigen in infected insect cells by immunofluorescence (see above). Consequently, an SDS boiling method was used and provided antigen which was suitable for detection by RIA. Under the conditions described in Materials and Methods, the rabbit anti-*vif* antibody yielded a binding ratio of greater than 2, indicative of specific binding. When AcNPV infected insect cells or uninfected insect cells were used as the antigen source low levels of binding were observed (data not shown).

Sera from 238 HIV-1 infected individuals were analysed for the presence of anti-*vif* antibodies using the RIA described above. Sixty samples were positive for anti-*vif* antibodies (25.3%). A sub-set of these sera also gave positive reactions against insect cells expressing *vif* in an immunofluorescence analysis (data not shown). The sera were derived from a broad spectrum of HIV-1 positive including asymptomatic and symptomatic and individuals with a wide range of CD4 levels (0–1,160 $\times$ 10^6 CD4 cells/L). The prevalence of anti-*vif* antibodies after segregation of the patients into there comparably sized groups comprising CD4 levels of 0–70 $\times$ 10^6 cells/L, 82–280 $\times$ 10^6 cells/L and 290–1160 $\times$ 10^6 cells/L is shown in Table I. There was a statistically significant increase in anti-*vif* antibodies in patients with CD4 levels in the two lower CD4 strata vs. the higher strata although there was no significant difference in the prevalence of anti-*vif* antibodies between patients in the two lower CD4 strata (0–70 $\times$ 10^6 and 82–280 $\times$ 10^6 cells/L). Subsequent analysis of the group according to the median CD4 level (155 $\times$ 10^6 CD4 cells/L) demonstrated that the prevalence of anti-*vif* antibodies was significantly higher in patients with lower CD4 cell numbers (P = 0.005).

The correlation of anti-*vif* antibodies with stage of HIV infection is shown in Table II. patients who had had an AIDS defining illness (symptomatic HIV infection) were more likely to have anti-*vif* antibodies (P = 0.0003). In contrast, when the patients were segregated according to the presence or absence of p24 antigen there was no difference in the prevalence of anti-*vif* antibodies (27% vs. 24.8%). These data are shown in Table III. As expected p24 antigenaemia was more prevalent in patients with symptomatic HIV infection (30/138 vs. 54/100; $P < 0.0001$). The overlap between p24 antigen positivity and presence of anti-*vif* antibodies is shown in Figure 3. Only 26 patients possessed both anti-*vif* antibodies and p24 antigenaemia, representing 43.5% of all patients who were anti-*vif* positive and 24.8% of patients who were p24 antigenaemic.

DISCUSSION

Recombinant baculovirus infected insect cells expressing the HIV-1 *vif* protein have been used as a substrate to screen sera from a broad spectrum of patients with HIV infection using a radioimmunoassay methodology. The results show that *vif* is immunogenic in vivo and that the proportion of patients who possess anti-*vif* antibodies increases with falling CD4 cell levels and is highest in patients whose CD4 cell level is less than 100 $\times$ 10^6 cells/L. These data are consistent with previous reports describing increasing prevalence of anti-*vif* antibodies in patients with AIDS and as HIV-1 infection progresses [Wieland et al., 1990; Schwander et al., 1992]. However, the absolute prevalence detected in the current study is lower than those previously reported. For example, in our study 37% of patients with symptomatic HIV disease were positive for anti-*vif* antibodies whereas Schwander et al. [1992] reported a seroprevalence of between 81% and 90% for *vif* antibodies in patients at a similar stage of HIV disease (CDC IV B/D and CDC IV A/C respectively). These differences may reflect the meth-

TABLE II. Prevalence of Anti-*vif* Antibodies in Patients With Asymptomatic or Symptomatic (CDCIV) HIV Infection

HIV disease category	Number in group	Anti-*vif* positive (%)
Symptomatic	100	37 (37)*
Asymptomatic	138	22 (15.9)*

*$P = 0.0003$.

TABLE III. Relationship Between Anti-*vif* Antibodies and p24 Antigenemia

p24 Antigenaemia	Number in group	Anti-*vif* positive (%)
Positive	96	26 (27)*
Negative	137	34 (24.8)*

HIV-1 p24 antigen results were available for 233 patients.
*P = not significant.

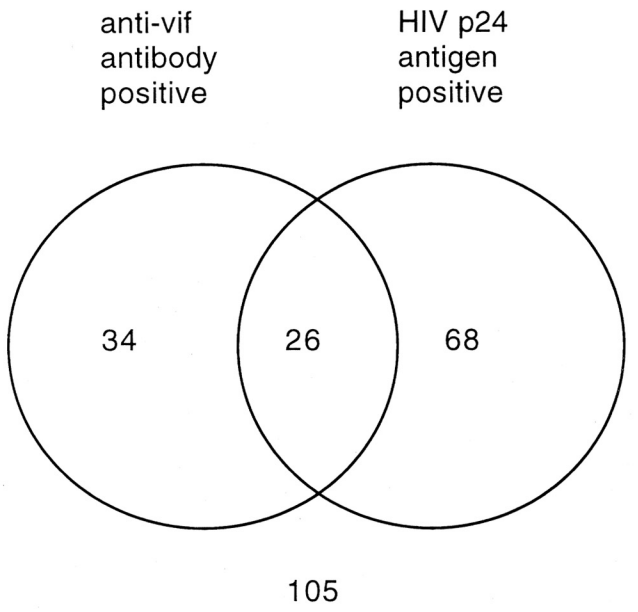

Fig. 3. Venn diagram showing the correlation between the presence of HIV p24 antigenemia and anti-*vif* antibodies. The value in each set represents samples derived from individual patients. HIV-1 p24 antigen results were available for 233 patients.

odologies used to determine the presence of anti-*vif* antibodies and/or the spectrum of patients chosen. In the current study patients comprised those with primary HIV-1 infection through to patients with AIDS. Consequently, the median CD4 count of the patients analysed was 155×10^6 cells/L. Since anti-*vif* seroprevalence was highest in patients with symptomatic disease and low CD4 levels, a simple explanation to account for these observations based upon HIV-1 load is attractive. In order to investigate this factor further, the patients were stratified according to the presence of p24 antigenaemia as an indirect measure of viral replication. The results did not support an explanation of increased anti-*vif* antibodies based solely upon increased viral replication with no significant difference in anti-*vif* seroprevalences between p24 antigen positive and negative individuals. To our knowledge, the lack of correlation between p24 antigenaemia and presence of anti-*vif* antibodies has not been previously reported. Since 41.2% of the samples were p24 antigen positive and p24 antigen was more likely to be present in patients with low CD4 counts and symptomatic disease an alternative explanation must exist to account for these data. Thus, it appears that anti-*vif* antibodies act as a marker of symptomatic HIV infection independent of p24 antigenaemia. It will be interesting to ascertain whether anti-*vif* antibodies are also independent of elevated viral load and their relationship to disease progression. The increased prevalence of anti-*vif* antibodies at late stages of HIV infection would argue that the immune system is still capable of mounting a strong humoral immune response to antigens even in the context of low levels and possibly functionally impaired T-helper cell responses. The prevalence of anti-*vif* antibodies may reflect a shift from HIV strains that disseminate via virus-cell spread to ones that are more likely to spread via cell-cell fusion. Such an explanation is consistent with the observation that the switch from non-syncytium producing viruses to syncytium inducing virus in vitro occurs at the later stages of HIV disease and may be related to poor prognosis irrespective of viral load [Tersmette et al., 1988; Keet et al., 1994; Kozal et al., 1994]. Experiments to investigate this possibility are currently underway in our laboratory.

ACKNOWLEDGMENTS

Part of this work was supported by the UK Medical Research Council.

REFERENCES

Arya SK, Gallo RC (1986): Three novel genes of human T-lymphotropic virus type III: Immune reactivity of their products with sera from acquired immune deficiency syndrome patients. Proceedings of the National Academy of Sciences of the United States of America 83:2209–2213.

Berry NJ, Grundy JE, Griffiths PD (1986): An improved radioimmunoassay method for the detection of IgG antibodies against cytomegalovirus. Journal of Virological Methods 13:343–350.

Berry NJ, Grundy JE, Griffiths PD (1987): Radioimmunoassay for the detection of IgG antibodies to herpes simplex virus and its use as a prognostic indicator of HSV excretion in transplant recipients. Journal of Medical Virology 21:147–154.

Blanc D, Patience C, Schulz TF, et al. (1993): Transcomplementation of VIF-HIV-1 mutants in CEM cells suggests that VIF affects late steps of the viral life cycle. Virology 193:186–192.

Borman AM, Quillent C, Charneau P, et al. (1995): Human immunodeficiency virus type 1 Vif-mutant particles from restrictive cells: Role of Vif in correct particle assembly and infectivity. Journal of Virology 69:2058–2067.

Darlington J, Super S, Patel K, Grundy JE, Griffiths PD, Emery VC (1991) Use of the polymerase chain reaction to analyse sequence variation within a major neutralising epitope of glycoprotein B (gp58) in clinical isolates of human cytomegalovirus. Journal of General Virology 72:1985–1989.

Devash Y, Reagan K, Wood D, et al. (1990): Antibodies against AIDS proteins. Nature 345:581.

Fisher AG, Ensoli B, Ivanoff L, et al. (1987): The sor gene of HIV-1 is

required for efficient virus transmission in vitro. Science 237:888–893.

Franchini G, Robert-Guroff M, Aldovini A, et al. (1987): Spectrum of natural antibodies against five HTLV-III antigens in infected individuals: Correlation of antibody prevalence with clinical status. Blood 69:437–441.

Gabuzda DH, Lawrence K, Langhoff E, et al. (1992): Role of *vif* in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. Journal of Virology 66:6489–6495.

Goncalves J, Jallepalli P, Gabuzda DH (1994): Subcellular localization of the Vif protein of human immunodeficiency virus type 1. Journal of Virology 68:704–712.

Hoglund S, Ohagen A, Lawrence K, et al. (1994): Role of *vif* during packing of the core of HIV-1. Virology 201:349–355.

Kan NC, Franchini G, Wong-Staal F, et al. (1986): Identification of HTLV-III/LAV sor gene product and detection of antibodies in human sera. Science 231:1553–1555.

Keet IP, Krol A, Koot M, et al. (1994): Predictors of disease progression in HIV-infected homosexual men with CD4+ cells $<200 \times 10(6)/L$ but free of AIDS-defining clinical disease. AIDS 8:1577–1583.

Kidd IM, Emery VC (1993): The use of baculoviruses as expression vectors. Applied Biochemistry and Biotechnology 42:137–159.

King LA, Possee RD (1995): "A Laboratory Guide." London: Chapman and Hall. The Baculovirus Expression System.

Kozal MJ, Shafer RW, Winters MA, et al. (1994): HIV-1 syncytium-inducing phenotype, virus burden, codon 215 reverse transcriptase mutation and CD4 cell decline in zidovudine-treated patients. Journal of Acquired Immune Deficiency Syndromes 7:832–838.

Lamhamedi-Cherradi S, Culmann-Penciolelli B, Guy B, et al. (1992): Qualitative and quantitative analysis of human cytotoxic T-lymphocyte responses to HIV-1 proteins. AIDS 6:1249–1258.

Lee TH, Coligan JE, Allan JS, et al. (1986): A new HTLV-III/LAV protein encoded by a gene found in cytopathic retroviruses. Science 231:1546–1549.

Matsuura Y, Possee RD, Overton HA, et al. (1987): Baculovirus expression vectors: The requirements for high level expression of proteins, including glycoproteins. Journal of General Virology 68:1233–1250.

Murphy G, Kavanagh T (1989): Speeding-up the sequencing of double-stranded DNA. Nucleic Acids Research 16:5198.

Oberste MS, Gonda MA (1992): Conservation of amino-acid sequence motifs in lentivirus Vif proteins. Virus Genes 6:95–102.

Sakai K, Ma XY, Gordienko I, et al. (1991): Recombinational analysis of a natural noncytopathic human immunodeficiency virus type 1 (HIV-1) isolate: Role of the *vif* gene in HIV-1 infection kinetics and cytopathicity. Journal of Virology 65:5765–5773.

Sakai H, Shibata R, Sakuragi J, et al. (1993): Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. Journal of Virology 67:1663–1666.

Sambrook J, Fritsch EF, Maniatis T (eds) (1989): "Molecular Cloning: A Laboratory Manual," 2nd ed. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

Schwander S, Braun RW, Kuhn JE, et al. (1992): Prevalence of antibodies to recombinant virion infectivity factor in the sera of prospectively studied patients with HIV-1 infection. Journal of Medical Virology 36:142–146.

Sodroski J, Goh WC, Rosen C, et al. (1986): Replicative and cytopathic potential of HTLV-III/LAV with sor gene deletions. Science 231:1549–1553.

Tersmette M, de Goede RE, Al BJ, et al. (1988): Differential syncytium-inducing capacity of human immunodeficiency virus isolates: Frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Journal of Virology 62:2026–2032.

von Schwedler U, Song J, Aiken C, et al. (1993): Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. Journal of Virology 67:4945–4955.

Wieland U, Kuhn JE, Jassoy C, et al. (1990): Antibodies to recombinant HIV-1 *vif*, tat, and nef proteins in human sera. Medical Microbiology and Immunology 179:1–11.

Wieland U, Kratschmann H, Kehm R, et al. (1991): Antigenic domains of the HIV-1 *vif* protein as recognized by human sera and murine monoclonal antibodies. AIDS Research and Human Retroviruses 7:861–867.